

Cover Page

BARD Project Number: IS-4473-11

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Project Title:

The therapeutic potential of *Af* and its active compounds for Alzheimer's disease

Investigators

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Science Center

Keywords Achillolide A, 3,5,4'-Trihydroxy-6,7,3'-trimethoxyflavone, Neuroinflammation, Oxidative stress, Phytochemicals, Neurodegenerative diseases

Abbreviations

A β – Amyloid beta

AcA – Achillolide A

AD - Alzheimer's disease

APP, sAPP, Amyloid precursor proteins, soluble APP

ELISA – Enzyme-linked immunosorbent assay

FAD - Familial Alzheimer's disease

PS1, PS2 – Presenilin-1, Presenilin-2

TTF - 3,5,4'-Trihydroxy-6,7,3'-trimethoxyflavone

Budget: IS: 173,000 \$

US: 147,000 \$

Total: 320,000 \$

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged			2	2
Submitted, in review, in preparation	3, In Prep			3
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts			2	2
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training:

Alona Telerman, identity number: 304374168

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1			1
Longer Visits (Sabbaticals)				

Description Cooperation:

The cooperation between the scientists working on this proposal was of a synergistic nature.

Dr. Ofir (Israel), who has experience in desert plant cultivation, was responsible for the cultivation of the different *Af* accessions. **Prof. Kashman** (Israel) who is an expert in the purification of natural substances and the determination of their structures purified AcA and TTF from *Af*. **Dr. Elmann** (Israel) who has expertise in oxidative stress and neuroinflammation was responsible for the cellular bioassays. **Dr. Lazarov** (USA) who has expertise in Alzheimer's disease and performed experiments in transgenic animal and cellular models of Familial Alzheimer's disease.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

We chose to focus our investigations on the effect of the active forms, TTF and AcA, rather than the whole (crude) extract.

1. To establish cultivation program designed to develop lead cultivar/s (which will be selected from the different *Af* accessions) with the highest yield of the active compounds **TTF** and/or **achillolide A (AcA)**. These cultivar/s will be the source for the purification of large amounts of the active compounds when needed in the future for functional foods/drug development. This task was completed.
2. To determine the effect of the *Af* extract, TTF and AcA on neuronal vulnerability to oxidative stress in cultured neurons expressing FAD-linked mutants. Compounds were tested in N2a neuroblastoma cell line. In addition, we have tested the effects of TTF and AcA on signaling events promoted by H₂O₂ in astrocytes and by β - amyloid in neuronal N2a cells.
3. To determine the effect of the *Af* extract, TTF and AcA on neuropathology (amyloidosis and tau phosphorylation) in cultured neurons expressing FAD-linked mutants.
4. To determine the effect of *Af* extract, AcA and TTF on FAD-linked neuropathology (amyloidosis, tau phosphorylation and inflammation) in transgenic mice.
5. To examine whether *Af* extract, TTF and AcA can reverse behavioral deficits in APPswe/PS1 Δ E9 mice, and affect learning and memory and cognitive performance in these FAD-linked transgenic mice.

Background to the topic. Neuroinflammation, oxidative stress, glutamate toxicity and amyloid beta (A β) toxicity are involved in the pathogenesis of Alzheimer's diseases. We have previously purified from *Achillea fragrantissima* two active compounds: a protective flavonoid named 3,5,4'-trihydroxy-6,7,3'-trimethoxyflavone (TTF, Fl-72/2) and an anti-inflammatory sesquiterpene lactone named achillolide A (AcA).

Major conclusions, solutions, achievements. In this study we could show that **TTF** and **AcA** protected cultured astrocytes from H₂O₂ –induced cell death via interference with cell signaling events. TTF inhibited SAPK/JNK, ERK1/2, MEK1 and CREB phosphorylation, while AcA inhibited only ERK1/2 and MEK1 phosphorylation. In addition to its protective activities, **TTF** had also anti-inflammatory activities, and inhibited the LPS-elicited secretion of the proinflammatory cytokines Interleukin 6 (IL-6) and IL-1 β from cultured microglial cells. Moreover, **TTF** and **AcA** protected neuronal cells from glutamate and A β cytotoxicity by reducing the glutamate and amyloid beta induced levels of intracellular reactive oxygen species (ROS) and via interference with cell signaling events induced by A β . These compounds also reduced amyloid precursor protein net processing *in vitro* and *in vivo* in a mouse model for Alzheimer's disease and improved performance in the novel object recognition learning and memory task.

Conclusion: TTF and AcA are potential candidates to be developed as drugs or food additives to prevent, postpone or ameliorate Alzheimer's disease.

Implications, both scientific and agricultural. The synthesis of AcA and TTF is very complicated. Thus, the plant itself will be the source for the isolation of these compounds or their precursors for synthesis. Therefore, *Achillea fragrantissima* could be developed into a new crop with industrial potential for the Arava-Negev area in Israel, and will generate more working places in this region.

Achievements

Significance of main scientific achievements or innovations.

This study provides preclinical evidence about the potential of two different phytochemicals, AcA and TTF, that were purified from the desert plant *Achillea fragrantissima* (Af), to be developed as drugs and/or functional foods for the prevention and/or amelioration of Alzheimer's disease.

We showed that AcA and TTF protected both astrocytes and neuronal cells from oxidative stress that was caused by different stressors: H₂O₂, sodium-nitroprusside, glutamate and amyloid beta. We also got insight into the mechanisms by which these compounds protect astrocytes and neuronal cells from oxidative stress and could show that their protective effect can be partially attributed to their inhibitory effect on the phosphorylation of MAPK. It should be noted that the effect of the sesquiterpene-lactone AcA and the flavonoid TTF on signaling events which are involved in the same stress (e.g. H₂O₂) were different, and for example, while TTF inhibited CREB phosphorylation (See attached article in the Journal of Neurochemistry), AcA had no effect on the phosphorylation of this transcription factor (data not shown). We also compared the activities of TTF and AcA to those of memantine and could show that these compounds share similar effects on several (but not all) signaling events. For example – while AcA (Fig. 8) and TTF (Fig. 21) inhibited A β -induced ERK1/2 phosphorylation in N2a cells, memantine did not have any effect. While AcA and memantine did not inhibit A β -induced MEK1 phosphorylation in N2a cells (Fig. 9), TTF inhibited MEK1 phosphorylation (Fig 19). Also in astrocytes, AcA, TTF, and memantine inhibited the H₂O₂ -induced phosphorylation of MEK1, ERK1/2 and SAPK/JNK, although to different extent. It should also be noted that AcA was better than memantine with respect to protection of N2a cells from glutamate toxicity (Fig. 3). Comparing these two compounds, AcA was a better anti-inflammatory compound than TTF, and TTF was a better protective compound than AcA, and protected astrocytes and neuronal cells to higher extent at lower concentrations. It is possible that because of the complementary activities of TTF and AcA and because of the multifactorial nature of neurodegenerative diseases, a combination of both compounds or even the whole extract of Af might have an advantage on the use of each compound separately. Based on that, a combination therapy of memantine and TTF or AcA may have an additive effect.

Importantly, we observed a direct effect of AcA and TTF on molecules linked to FAD. Specifically, the familial form of Alzheimer's disease is caused by mutations in amyloid precursor protein (APP). One important effect of the mutations is the enhanced amyloidogenic

processing of APP. We observed that AcA and TTF stabilize APP, i.e., reduce its rate of processing. This was manifested by increased full length APP and no change in the processed form sAPP, suggesting that APP is stabilized on the cell membrane. This important observation was reproduced in cell culture and in transgenic mice harboring FAD-linked APP^{swe}/PS1 Δ E9 mutants. Lastly, our preliminary results show that AcA may have a positive effect on learning and memory in the APP^{swe}/PS1 Δ E9 mice.

In summary, we successfully achieved our original objectives and provide evidence suggesting that AcA and TTF modulate APP processing, and may improve aspects of learning and memory in transgenic mouse model FAD-linked APP^{swe}/PS1 Δ E9. In addition, we provide a cultivation protocol of the lead cultivar, which contains the highest amounts of AcA and TTF. Lastly, we got seeds of the lead cultivar.

Agricultural and/or economic impacts of the research findings, if known.

Af which is a desert plant, could be developed into a new crop with industrial potential, that will be an important contribution for the Arava-Negev area in Israel (as well as other desert areas), the most suitable place to raise *Af*, and will generate more working places in this region. The farmers in this area together with their children and new settlers are most interested in new crops, since their income stems from agriculture. It is estimated that in the USA alone, there are 5 million people affected with Alzheimer's disease (out of 20 million people worldwide), and this number is expected to triple in the next ten years. Medicare cost of Alzheimer's disease is estimated by \$148 billion a year. Therefore, a therapy for the disease is a priority for the American government and for the sake of the affected families. Alzheimer's patients might benefit from the research and development of AcA and/or TTF as drugs or a food additive for the prevention and/or treatment of Alzheimer's disease.

Details of cooperation: The achievements described above and below and the execution of the project objectives would not be possible without this collaboration. The cooperation between the scientists working on this proposal was of a synergistic nature: **Dr. Rivka Ofir** (Israel), who has experience in desert plant cultivation, was responsible for the cultivation of the different *Af* accessions. **Prof. Yoel Kashman** (Israel) who is an expert in the purification of natural substances and the determination of their structures purified the active compounds from *Af*. **Dr. Anat Elmann** (Israel) who has expertise in oxidative stress and neuroinflammation was responsible for the cellular bioassays. **Dr. Orly Lazarov** (USA) who has expertise in Alzheimer's disease and transgenic animal models of Familial Alzheimer's disease performed the *in vivo* experiments.

List of Publications:

1. **Elmann, A.**, Telerman, A., Mordechay, S., Erlank, H., Rindner, M., **Ofir, R.** and **Kashman, Y.** (2014). 3,5,4'-Trihydroxy-6,7,3'-trimethoxyflavone protects astrocytes against oxidative stress via interference with cell signaling and by reducing the levels of intracellular reactive oxygen species. *Neurochemistry International*: 78:67-75.
2. **Anat Elmann**, Alona Telerman, Sharon Mordechay, Hilla Erlank, Miriam Rindner, **Yoel Kashman** and **Rivka Ofir** (2015). Down-regulation of microglial activation by achillolide A. *Planta Medica*, 81(3):215-221.